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Research Article

Evaluation of Invitro Antioxidant Activity of Flowers of *Tagetes erecta*

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ABSTRACT

Tagetes erecta (*T. erecta*) is an herbaceous species, having wide ethno medicinal and traditional uses. It demonstrates different pharmacological activities like antioxidant, antimicrobial and anti depressant. The present study deals with the estimation of antioxidant properties of four solvent extracts of flowers of *T. erecta*. The study aims to discover potent extracts as a novel source of natural antioxidant. The crude extracts; Petroleum ether (PE), Chloroform (CF), Ethyl Acetate (EA) and Methanol (MET) were screened for their free radical scavenging capacity and reducing powers using DPPH, Superoxide and Reducing power assay. Ascorbic acid was used as standard. All the extracts show considerable antioxidant potential. However, methanolic extract can strongly scavenge DPPH and superoxide radical with significant IC_{50} value range 30.08 ± 0.98 µg/mL and 64.22±0.04 µg/mL respectively. The IC_{50} value of the extracts follows the order; MET<EA<PE<CF. Lowest IC_{50} value indicates highest antioxidant potential. The reducing power of extracts were in the order; CF<PE<EA<Met.

Keywords: *Tagetes erecta*, Antioxidant activity, DPPH (1, 1-diphenyl-2-picrylhydrazyl), Reducing power, Superoxide.

INTRODUCTION

Chronic diseases contribute almost 60% of the total deaths reported worldwide and its burden is increasing rapidly[1]. Most of the disorders in humans are caused by reactive free radicals. They are defined as a molecular fragment containing one or more unpaired electron in the outermost shell. They can exist independently and are more reactive than non radical species. They are formed by homolytic cleavage, highly reactive and can start a chain reaction. They are ROS and RNS which includes radicals such as superoxide (O^{2-}), hydroxyl (OH-), peroxyl (RO^{2-}), hydroperoxyl (HO^{2-}), alkoxyl (RO^{-}), peroxyl (ROO-), nitric oxide (NO-), nitrogen dioxide (NO^{2-}) and lipid peroxyl (LOO-); and non radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($1\Delta g$), peroxynitrate ($ONOO^{-}$), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH). They have a twofold function in our body, both harmful and beneficial[2]. Over production of free radicals causes degradation of biomolecules i.e. lipid peroxidation, oxidation of DNA, degradation of protein, which causes various chronic disorders[1].

Oxidation reactions involve transfer of electron from one compound to another or addition of oxygen[3]. ROS, reactive oxygen species are potentially reactive forms of oxygen which are continuously produced inside the body. To fight against these, a group of compounds called antioxidants are known. Antioxidants are substances that prevent oxidative damage, promoted by oxygen or peroxides. They scavenge reactive oxygen or nitrogen species (ROS/RNS) to stop chain reactions[4,5].

A discrepancy between antioxidants and free radicals results in oxidative stress leading to cellular damage. This oxidative damage is an important factor involved in several unrelenting human diseases like diabetes mellitus, arthritis, cancer, atherosclerosis neurodegenerative disorders and in ageing process also[6,7]. It has been studied in several researches that a diet rich in antioxidants may help prevent some of this damages[7]. Herbal medicines have been used since ages for the treatment of various ailments. Drugs are made from seeds, leaves, bark, fruits, flowers and other plant parts. It can be an alternative and valuable source for drug discovery[7,8].

T. erecta is an herbaceous species, belongs to family Compositae or Asteracea. The plant is native to Mexico, North America, South America and naturalised in tropics and sub tropics including India and Bangladesh[9]. It is also known as Americam Marigold or African Marigold. It is a popular garden plant, having ornamental value and a wide application in herbal remedy. Its different species have been found to show different activities like antibacterial, anti depressant, anti inflammatory, antimycotic, larvacidal, insecticidal, mosquitocidal, and nematicidal activity[10].

Its flower is choice of medication in many cases[11]. The plant has been found to contain various secondary metabolites which show numerous pharmacological activities. It includes Flavonoids, Carotenoids, Polyphenol, Lutein, Xanthophylls, Essential oils, etc.[12]

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Being rich source of many bioactive components and wide availability, *T. erecta* is now one of the prime targets of researchers working on chemistry of natural products.

The major pigments present in *T. erecta* are basically Flavonoids and Carotenoids[13].

Some of the isolated components from plant are quercetagetin, quercitin, phenolics, synergic acid, methyl-3,5-dihydroxy-4-methoxy benzoate, thienyl and ethyl gallate[9].

The fatty acids present in marigold flower are linoleic acid, palmitic acid and oleinic acid. The dominant antioxidant compounds are Lutein esters, tocopherol, β-tocopherol, γ-tocopherol and δ- tocopherol[14]. Lutein, a natural antioxidant, is the major pigment present in *Tagetes* species. It is a yellow coloured oxycarotenoid having two cyclic end groups and a basic C 40 isoprene unit having molecular formula $C_{40}H_{56}O_2$[10,15]. In the present study, antioxidant potential of different extracts of flowers of *T. erecta* was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, Superoxide scavenging and Reducing power assay.

MATERIAL AND METHODS

Plant collection

The flowers of *T. erecta* were collected from local market of Bhopal (Madhya Pradesh, India).The plant was identified and authenticated by Dr. Zia ul Hasan, HOD, Department of Botany, Saifia College, Bhopal. The voucher specimen number is 518/Bot/Saifia/2015.

Preparation of plant extracts

The flowers of *T. erecta* were shade dried for 7-10 days. After that they were dried in oven at a temperature less than 60˚ C in order to remove the moisture content. The plant material is then grinded in a mechanical grinder to make a fine powder. The material was extracted by maceration process, with solvents of increasing polarity, sequentially from Petroleum ether, Chloroform, Ethyl Acetate and Methanol respectively. The fractions were evaporated to dryness and stored for further investigation[16].

Chemicals required

1, 1-diphenyl-2-picrylhydrazyl (DPPH), Methanol, Potassium ferricyanide (1%W/V),Phosphate buffer (0.2 M, pH 6.6), Trichloroacetic acid solution (10% W/V), Ferric chloride (0.1% W/V), NBT(Nitro blue tetrazolium), alkaline DMSO (Dimethyl sulfoxide).

Experimental Procedure

Antioxidant capacity of extracts of *T. erecta* flowers was determined by DPPH radical scavenging activity, Reducing power assay and Superoxide assay.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity[17]

Different concentrations (20, 40, 60, 80, 100µg/mL) of plant extracts and 0.1mM DPPH solution was prepared in methanol. Ascorbic acid (100µg/mL) was used as standard. 2mL of DPPH solution and 1 mL of methanol was used as control. 2 mL of plant extract of various concentrations was mixed with 2 mL of DPPH solution and control separately. The mixture was incubated for 10 minutes in the dark and absorbance was measured at 515 nm by spectrophotometer using methanol as blank. The percentage inhibition of DPPH radical was calculated by following formulae; % Inhibition= $[(A_{control} - A_{sample})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. IC_{50} was calculated by plotting % inhibition as a function of sample concentration. IC_{50} is defined as extract concentration necessary to inhibit 50% of DPPH solution.

Reducing power assay[18]

Different concentrations (20, 40, 60, 80, 100µg/mL) of plant extract and standard were prepared. 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium ferricyanide (1%W/V) were added to 0.5 mL of sample concentrations. Reaction mixture was incubated for 20 min at 50° C. After cooling, reaction was terminated by adding 1.5 mL of trichloroacetic acid (10%, w/v). 0.5 mL ferric chloride (0.1% W/V) was added and absorbance was measured at 700 nm. Reducing power is calculated by plotting a curve between absorbance and concentration. Increased absorbance of the reaction mixture indicates increase in reducing power. The tests were performed in triplicates using ascorbic acid as reference standard. Furthermore, the reducing power of plant extracts was expressed as Ascorbic acid equivalent (AAE) microgram per mL of compound.

Superoxide scavenging assay[19, 20]

To the reaction mixture containing 0.1 mL of NBT (Nitro blue tetrazolium), 0.3 mL of extract and 1 mL of alkaline DMSO (Dimethyl sulfoxide) was added to give a final volume of 1.4 mL and the absorbance was measured at 560 nm. Plain DMSO used as blank and reaction mixture without extract (water in place of extract) used as control. The superoxide radical scavenging capacity was calculated by the following formula:

Superoxide scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test extract. IC_{50} was calculated by plotting % inhibition as a function of sample concentration. The results were compared with the reference antioxidant ascorbic acid.

Statistical Analysis

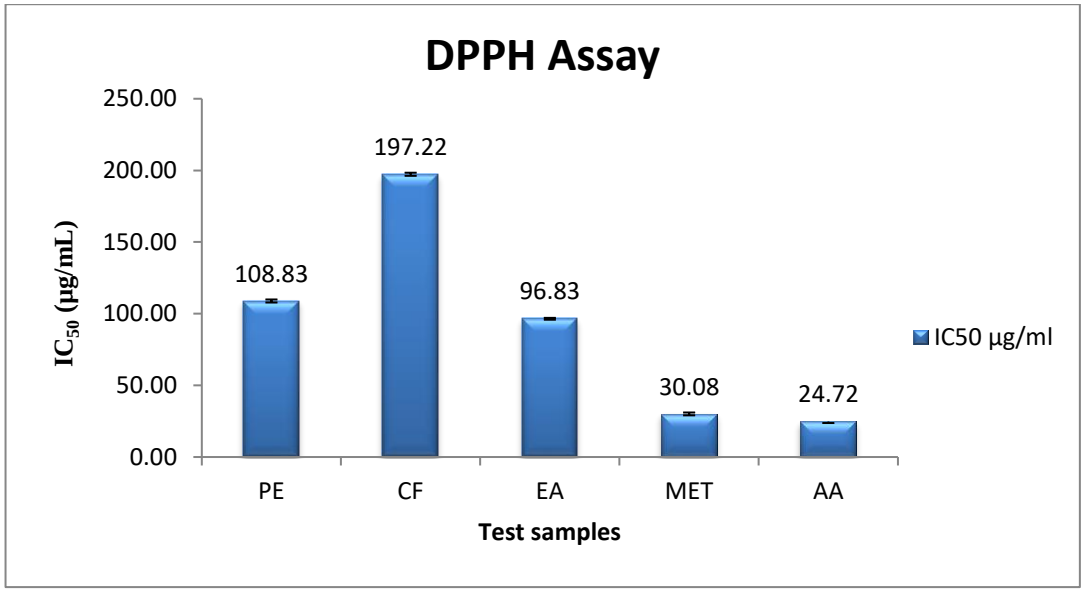
All the assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

RESULTS AND DISCUSSSION

The flower extracts of *T. erecta* were subjected to *in vitro* tests and their antioxidant activities was evaluated by DPPH radical-scavenging, Reducing power, Superoxide scavenging assay. Ascorbic acid (AA) was used as standard reference compound for all tests.

DPPH Radical Scavenging Activity

DPPH is a stable free radical, purple in color. It decolorizes on accepting an electron in presence of antioxidant molecule. This is measured by spectrophotometer from the changes in absorbance at 515 nm[21]. The reduction ability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants[22]. All the extracts of *T. erecta* showed a good inhibitory activity against DPPH radical. The scavenging activity of extracts and standard on the DPPH radical expressed as IC_{50} values



Graph 1: DPPH scavenging capacity of plant extracts of *T. erecta* flowers.

Table 1: DPPH radical scavenging activity of plant extracts of *T. erecta* flowers.

T. erecta extract	IC50 (µg/ mL AAE)
PE	108±1.09
CF	197.22±1.16
EA	96.83±0.26
MET	30.08±0.98
AA (Std)	24.72±0.36

Table 2: Superoxide scavenging activity of plant extracts of *T. erecta* flowers.

T. erecta extract	IC50 (µg/ mL AAE)
PE	88.51±0.19
CF	116.84±0.09
EA	80.36±0.33
MET	64.22±0.04
AA (Std)	59.75±0.02

and follows the order; AA (24.72µg/mL) MET (30.08µg/mL), EA (96.83µg/mL), PE (108.83µg/mL), CF (197.22µg/mL). Highest quenching ability is shown by methanol extract while chloroform extract showed lowest scavenging activity. The experimental data reveal that polar extracts have stronger free radical scavenging effect than the non polar ones. IC_{50} value of Methanolic extract is close to ascorbic acid which is a well known antioxidant.

DPPH radical scavenging activity expressed as Ascorbic acid equivalent (AAE), PE=Pet Ether, CF=Chloroform, EA= Ethyl acetate, MET=Methanol, AA=Ascorbic acid as standard. Values are expressed as mean ± standard deviation.

Reducing power assay

The antioxidant activity of plant extracts is due to polyphenols present in them which show redox properties. These are important since they decompose peroxides, neutralize free radicals, quench singlet and triplet oxygen. [21] In this assay**,** conversion of the Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrocyanide complex occurs due to presence of reducers. The yellow color of the test sample changes to different shades of green and blue depending on the reducing power of each compound. This color change is measured at 700 nm by spectrophotometer[23]. The reducing power of extracts is shown graphically by depicting absorbance as a function of concentration. The reducing power of all the extracts increased with increase in concentration. The reducing ability of the extracts shows the order; CF<PE<EA<MET. Reducing power of methanol extract is highest which is comparable to standard compound ascorbic acid. PE has higher reducing ability than CF due to presence of Carotenoids and xanthophylls which are potent antioxidants.

Superoxide scavenging assay

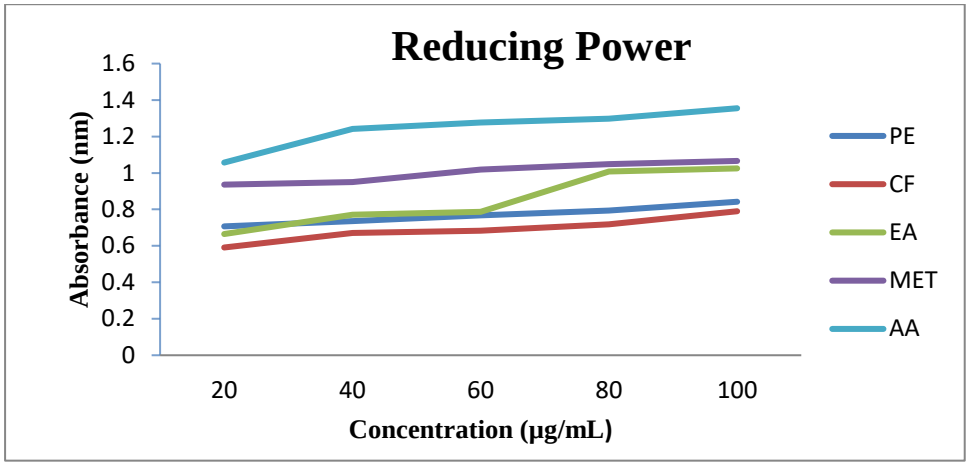
Superoxide is generated in vivo by numerous oxidative enzymes, including xanthine oxidase. Superoxide anions were generated in vitro enzymatically that reduces NBT and forms a blue colored chromophore, which can be measured at 560 nm by spectrophotometer. As concentration of extracts is increased there is decrease in absorbance due to the antioxidants present in the extract. This indicates the consumption of superoxide anion in the reaction mixture[24,25].

Superoxide radical scavenging activity of crude extracts follows the order; AA (59.75±0.02), MET (64.22±0.04), EA (80.36±0.33), PE (88.51±0.19), CF (116.84±0.09). The experimental data shows that methanolic extract have a noticeable effect in scavenging superoxide radical because of their higher total Phenolics and flavonoids contents.

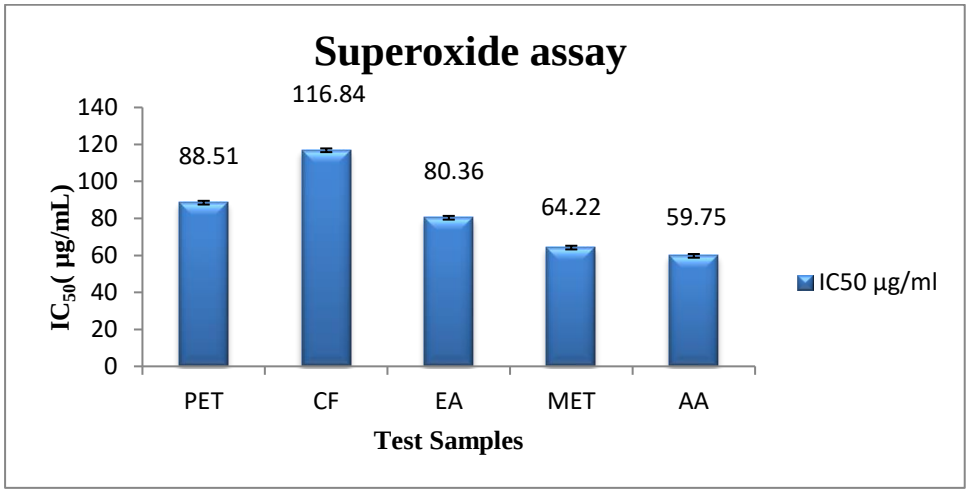
Superoxide scavenging activity expressed as Ascorbic acid equivalent (AAE), PE=Pet Ether, CF=Chloroform, EA= Ethyl acetate, MET=Methanol, AA=Ascorbic acid as standard. Values are expressed as mean ± standard deviation**.**

CONCLUSION

The data presented here shows that flowers of *T. erecta* extracts have a significant antioxidant activity close to that of standards and may be used as an alternative to the



Graph 2: Reducing capacity of plant extracts of *T. erecta* flowers.



Graph 3: Superoxide scavenging capacity of plant extracts of *T. erecta* flowers.

synthetic antioxidants. Thus, this study gives a strong impact for expanding the investigations of natural antioxidants. Highest level is observed in methanolic extract as compared to other tested extracts and is also capable of reducing reactive oxygen species. The present article revealed that *T. erecta* is a significant medicinal plant and a potent antioxidant. Further studies should be carried out for isolation and characterization of bioactive compounds from *T. erecta* which independently or in adjunct are responsible for showing antioxidant activity.

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REFERENCES

1. Jayathilake C, V. Rizliya, Liyanage R. Antioxidant and free radical scavenging capacity of extensively used medicinal plants in Sri Lanka. Procedia Food Science 2016; 6:123-6.
2. Mahantesh S.P, Gangawane A.K, Patil C.S. Free radicals, antioxidants, diseases and phytomedicines in human health: future perspects. World Res J Med Aromat Plant 2012; 1(1):6-10.
3. Gupta VK, Sharma SK. Plant as Natural Antioxidant,Nat. Prod.Rad 2006; 5(4):326-34.
4. Huang D, Ou B, Ronald L. Prior. The Chemistry behind Antioxidant Capacity Assays. J. Agric. Food Chem. 2005**;** 53:1841-56.
5. Patel VR, Patel PR, Kajal SS. Antioxidant Activity of some medicinal plant in western region of India. Adv Biol Res 2010; 4 (1): 23-6.
6. Nooman A K, Ashok K Shakya, Atif AL Othman, Zaha EL Agbar, Husni F. Antioxidant Activity of Some Common Plants. Turk J Biol 2008; 32: 51-5.
7. Li XZ, Zhong SN, Liu SM, Lu F. Recent Advances in Herbal Medicines treating Parkinson's disease. Fitoterapia 2013; 84:273-85.
8. Dela torre G.L.T, Arollado E.C, Atienza A.A, Manalo R.A.M. Evaluation of antioxidant capacity and identification of bioactive compounds of crude methanol extracts of *Caesalpinia pulcherrima* (L.) Swartz. Indian J Pharm Sci 2017;79(1): 113-23.
9. Kadam P V, Bhingare C L, Sumbe R B, Nikam R Y, Patil M J. Pharmacognostic, Physiochemical and Phytochemical Investigation of *Tagetes erecta* Linn flowers (Asteraceae). JBSO 2013; 1(1):21-4.

10. Dixit P, Tripathi S, Verma K N. A brief Study on Marigold *(Tagetes species).* Int. Res. J. Pharm. 2013; 4(1):43-8.
11. Nikkon F, Habib M R, Saud Z A, Karim M R. *Tagetes erecta* Linn. and its mosquitocidal potency against Culex quinquefasciatus. Asian Pac J Trop Biomed 2011; 1(3):186-8.
12. Valyova M, Stoyanov S, Markovska Y, Ganeva Y. Evaluation of *in-vitro* Antioxidant Activity and Free Radical Scavenging Potential of Variety of *Tagetes erecta* L. Flowers growing in Bulgaria. Int. J. Appl. Res. Nat. Prod. 2012; 5(2):19-25.
13. Devika R, Justin K. Screening and evaluation of bioactive components of *Tagetes erecta L.* by GC – MS Analysis. *Asian* J Pharm Clin Res 2014; 7(2):58-60.
14. Gong Y, Plander S, Xu H, Simandi B, Gao Y. Supercritical CO_2 extraction of oleoresin from marigold (*Tagetes erecta* L.) flowers and determination of its antioxidant components with online HPLC-ABTS (·+) assay. J. Sci. Food Agric.2011; 91(15):2875-81.
15. Pratheesh V B, Benny N, Sujatha C H. Isolation, Stabilization and Characterization of Xanthophyll from Marigold Flower-*Tagetes erecta-L*. Mod Appl Sci. 2009; 3(2):19-27.
16. Nishaa.S, Vishnupriya.M, Sasikumar.J.M, Hephzibah P Christabel, Gopalakrishnan.V.K . Antioxidant activity of Ethanolic extract of *Maranta arundinacea* L.tuberous rhizomes. Asian J Pharm Clin Res. 2012; 5(4):85-8.
17. Gulcin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). Eur Food Res Technol 2006; 223: 759-67.
18. R Jain and SK Jain, Total Phenolic Contents and Antioxidant Activities of Some Selected Anticancer Medicinal Plants from Chhattisgarh State, India. PhOL 2011; 2: 755-62.
19. Patil AP, Patil VV, Patil VR, In-vitro free radicals scavenging activity of Madhuca indica Gel. PhOL. 2009; 2:1344-52.
20. Yerra R, Senthikumar GP, Gupta M, Studies on in vitro antioxidant activities of methonal extracts of Mucuna pruriens (Fabaceae) seeds. European bulletin of drug research, 2005; 1: 31-9.
21. Saha M.R., Hasana S.M.R, Aktera R, Hossaina M,M, Alamb M.S, , Alam M.A and M. E. H. Mazumderc M.E.H. Bangl. J. Vet. Med 2008;6 (2): 197–202.
22. Rigane G, Ben Younes, S, Ghazghazi H, Ben, Salem, R. Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. Int Food Res J, 2013; 20(6): 3001-7.
23. Jayanthi P, Lalitha P. Reducing power of the solvent extracts of *Eichhornia crassipes* (mart.) Solms, Int J Pharm Pharm Sci 2011; 3(3):126-8.
24. Chidambaram U, Pachamuthu V, Natarajan S Elango B, Suriyanarayanan, Ramkumar K.M In vitro evaluation of free radical scavenging activity of Codariocalyx motorius root extract. Asian Pac J Trop Med 2013; 6(3): 188-194.
25. Gulchin I, Alici H.A, Cesur M. Chem. Pharm. Bull.2005; 53(3): 281-5.